



Amendments to the Claims

1. (Original) An embryonic stem cell line derived from a nucleus-transferred oocyte prepared by transferring a nucleus of a human somatic cell into an enucleated human oocyte.

2. (Original) The embryonic stem cell line of claim 1, which is a cell line deposited under the accession number of KCLRF-BP-00092.

3. (Currently amended) A method for preparing ~~an embryonic stem cell line~~ a blastocyst derived from a human somatic cell and a human oocyte, comprising ~~the steps of:~~

(1) culturing a human somatic cell to prepare a nuclear donor cell;

(2) enucleating a human oocyte to prepare a recipient oocyte;

(3) preparing a nucleus-transferred oocyte by transferring a nucleus of the nuclear donor cell into the recipient oocyte and fusing the nucleus of the nuclear donor cell and the recipient oocyte; and

(4) subjecting the nucleus-transferred oocyte to reprogramming, activation and *in vitro* culturing to form a blastocyst; ~~and~~

~~(5) isolating an inner cell mass from the blastocyst and culturing the inner cell mass in an undifferentiated state to establish the embryonic stem cell line.~~

4. (Currently amended) The method of claim ~~[[3]]~~45, wherein the embryonic stem cell line is a cell line deposited under the accession number of

KCLRF-BP-00092.

5. (Currently amended) The method of claim ~~[[3]]45~~, wherein the reprogramming in step (4) is conducted for a time period of up to 20 hours.

6. (Currently amended) The method of claim ~~[[3]]45~~, wherein the reprogramming in step (4) is conducted for a time period of up to 6 hours.

7. (Currently amended) The method of claim ~~[[3]]45~~, wherein the reprogramming in step (4) is conducted for a time period of up to 3 hours.

8. (Currently amended) The method of claim ~~[[3]]45~~, wherein the reprogramming in step (4) is conducted for a time period of about 2 hours.

9. (Currently amended) The method of claim ~~[[3]]45~~, wherein the activation in step (4) is performed by treating the nucleus-transferred oocyte with a calcium ionophore and subsequently with 6-dimethylaminopurine.

10. (Original) The method of claim 9, wherein the concentration of the calcium ionophore ranges from 5 μ M to 15 μ M.

11. (Original) The method of claim 9, wherein the concentration of the calcium ionophore is about 10 μ M.

12. (Original) The method of claim 9, wherein the concentration of 6-dimethylaminopurine ranges from 1.5mM to 2.5mM.

13. (Original) The method of claim 9, wherein the concentration of 6-dimethylaminopurine is about 2.0mM.

14. (Currently amended) The method of claim ~~[[3]]45~~, wherein the *in vitro* culturing in step (4) is performed by sequentially using at least two media, each having a different composition from the other.

15. (Original) The method of claim 14, wherein the *in vitro* culturing is performed by sequentially using two media having different compositions each other.

16. (Original) The method of claim 15, wherein the *in vitro* culturing is performed by sequentially using the G1.2 medium and the SNUnt-2 medium.

17. (Currently amended) The method of claim ~~[[3]]45~~, wherein step (4) is performed by reprogramming the nucleus-transferred oocyte for a time period of up to 20 hours, treating the nucleus-transferred oocyte with a calcium ionophore at a concentration ranging from 5μM to 15μM and subsequently with 6-dimethylaminopurine at a concentration ranging from 1.5mM to 2.5mM, and sequentially culturing the nucleus-transferred oocyte *in vitro* in the G1.2 medium and the SNUnt-2 medium.

18. (Currently amended) The method of claim ~~[[3]]~~45, wherein the inner cell mass is isolated from the blastocyst in step (5) by a process comprising the steps of:

- (1) removing the zona pellucida or part thereof from the blastocyst; and
- (2) isolating the inner cell mass by removing the trophoblast from the resulting blastocyst.

19. (Currently amended) The method of claim ~~[[3]]~~45, wherein the inner cell mass is cultured in step (5) on a feeder layer comprising a cell differentiated from the embryonic stem cell line of claim 1.

20. (Original) A neuro progenitor differentiated from an embryonic stem cell line derived from a nucleus-transferred oocyte prepared by transferring a nucleus of a human somatic cell into an enucleated human oocyte.

21. (Original) The neuro progenitor of claim 20, wherein the embryonic stem cell line is a cell line deposited under the accession number of KCLRF-BP-00092.

22. (Currently amended) A method for preparing the neuro progenitor of claim 20, comprising ~~the steps of~~:

- (1) culturing the embryonic stem cell line to form an embryoid body;
- (2) culturing the embryoid body in the presence of an agent suitable for

differentiating a cell of the embryoid body into the neuro progenitor; and

(3) selecting a cell expressing a marker of the neuro progenitor and culturing the selected cell to obtain the neuro progenitor.

23. (Original) The method of claim 22, wherein the embryonic stem cell line is a cell line deposited under the accession number of KCLRF-BP-00092.

24. (Original) The method of claim 22, wherein the agent employed in step (2) is selected from the group consisting of retinoic acid; ascorbic acid; nicotinamide; N-2 supplement; B-27 supplement; and a mixture of insulin, transferrin, sodium selenite and fibronectin.

25. (Original) A medium for use in carrying out the *in vitro* culturing in step (4) of claim 3, comprising:

95 to 110mM NaCl; 7.0 to 7.5mM KCl; 20 to 30mM NaHCO₃; 1.0 to 1.5mM NaH₂PO₄; 3 to 8mM sodium lactate; 1.5 to 2.0mM CaCl₂·2H₂O; 0.3 to 0.8mM MgCl₂·6H₂O; 0.2 to 0.4mM sodium pyruvate; 1.2 to 1.7mM fructose; 6 to 10mg/ml human serum albumin; 0.7 to 0.8μg/ml kanamycin; 1.5 to 3% essential amino acids; 0.5 to 1.5% nonessential amino acids; 0.7 to 1.2mM L-glutamine; and 0.3 to 0.7% a mixture of insulin, transferrin and sodium selenite.

26. (Currently amended) The medium of claim 25, ~~which comprises~~ comprising:

99.1 to 106mM NaCl; 7.2mM KCl; 25mM NaHCO₃; 1.2mM NaH₂PO₄; 5mM

sodium lactate; 1.7mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 0.5mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 0.3mM sodium pyruvate; 1.5mM fructose; 8 mg/ml human serum albumin; 0.75 $\mu\text{g/ml}$ kanamycin; 2% essential amino acids; essential amino acids; L-glutamine; and mixture of insulin, transferrin and sodium selenite.

27. (New) The method according to claim 3, wherein the step (2) comprises incising a part of zona pellucida of the oocyte and removing the cytoplasm containing first polar body by pressing the oocyte.

28. (New) The method according to claim 27, wherein the step (2) further comprises removing surrounding cumulus cells from the oocyte before the incising and the removing.

29. (New) The method according to claim 3, wherein the step (2) comprises holding said human oocyte with a holding pipette; incising a part of zona pellucida of the oocyte with an incision pipette; removing first polar body and nucleus from the oocyte supported by the holding pipette through a hole made by the incision process by pressing the oocyte with the incision pipette.

30. (New) The method according to claim 3, wherein the step (2) comprises removing part of cytoplasm containing first polar body corresponding to 10 to 15 percent of total cytoplasm.

31. (New) The method according to claim 3, wherein the reprogramming in step (4) is conducted for a time period of up to 20 hours.

32. (New) The method according to claim 3, wherein the reprogramming in step (4) is conducted for a time period of up to 6 hours.

33. (New) The method according to claim 3, wherein the reprogramming in step (4) is conducted for a time period of up to 3 hours.

34. (New) The method according to claim 3, wherein the reprogramming in step (4) is conducted for a time period of up to 2 hours.

35. (New) The method according to claim 3, wherein the activation in step (4) is performed by treating the nucleus-transferred oocyte with a calcium ionophore and subsequently with 6-dimethylaminopurine.

36. (New) The method of claim 35, wherein the concentration of the calcium ionophore ranges from 5 μ M to 15 μ M.

37. (New) The method of claim 36, wherein the concentration of the calcium ionophore is about 10 μ M.

38. (New) The method of claim 35, wherein the concentration of 6-dimethylaminopurine ranges from 1.5mM to 2.5mM.

39. (New) The method of claim 38, wherein the concentration of 6-dimethylaminopurine is about 2.0mM.

40. (New) The method of claim 3, wherein the *in vitro* culturing in step (4) is

performed by sequentially using at least two media, each having a different composition from the other.

41. (New) The method of claim 40, wherein the *in vitro* culturing is performed by sequentially using two media having different compositions from each other.

42. (New) The method of claim 41, wherein the *in vitro* culturing is performed by sequentially using G1.2 medium and SNUnt-2 medium.

43. (New) The method of claim 3, wherein step (4) is performed by reprogramming the nucleus-transferred oocyte for a time period of up to 20 hours, treating the nucleus-transferred oocyte with a calcium ionophore at a concentration ranging from 5 μ M to 15 μ M and subsequently with 6-dimethylaminopurine at a concentration ranging from 1.5mM to 2.5mM, and sequentially culturing the nucleus-transferred oocyte *in vitro* in G1.2 medium and SNUnt-2 medium.

44. (New) A blastocyst prepared by the method according to claim 3.

45. (New) A method for preparing an embryonic stem cell comprising:

(1) culturing a human somatic cell to prepare a nuclear donor cell;

(2) enucleating a human oocyte to prepare a recipient oocyte;

(3) preparing a nucleus-transferred oocyte by transferring a nucleus of the nuclear donor cell into the recipient oocyte and fusing the nucleus of the nuclear donor cell and the recipient oocyte;

(4) subjecting the nucleus-transferred oocyte to reprogramming, activation and *in vitro* culturing to form a blastocyst; and

(5) isolating an inner cell mass from the blastocyst and culturing the inner cell mass in an undifferentiated state to establish the embryonic stem cell line.

46. (New) The method according to claim 45, wherein the step (2) comprises incising a part of zona pellucida of the oocyte and removing the cytoplasm containing first polar body by pressing the oocyte.

47. (New) The method according to claim 44, wherein the step (2) further comprises removing surrounding cumulus cells from the oocyte before the incising and the removing.

48. (New) The method according to claim 45, wherein the step (2) comprises holding said human oocyte with a holding pipette; incising a part of zona pellucida of the oocyte with an incision pipette; removing first polar body and nucleus from the oocyte supported by the holding pipette through a hole made by the incision process by pressing the oocyte with the incision pipette.

49. (New) The method according to claim 45, wherein the step (2) comprises removing part of cytoplasm containing first polar body corresponding to 10 to 15% of total cytoplasm.

50. (New) A medium for use in carrying out the *in vitro* culturing in step (4) of claim 45, comprising:

95 to 110mM NaCl; 7.0 to 7.5mM KCl; 20 to 30mM NaHCO₃; 1.0 to 1.5mM NaH₂PO₄; 3 to 8mM sodium lactate; 1.5 to 2.0mM CaCl₂·2H₂O; 0.3 to 0.8mM MgCl₂·6H₂O; 0.2 to 0.4mM sodium pyruvate; 1.2 to 1.7mM fructose; 6 to 10 mg/ml

human serum albumin; 0.7 to 0.8 $\mu\text{g/ml}$ kanamycin; 1.5 to 3% essential amino acids; 0.5 to 1.5% nonessential amino acids; 0.7 to 1.2mM L-glutamine; and 0.3 to 0.7% a mixture of insulin, transferrin and sodium selenite.

51. (New) A stem cell line prepared by the method according to claim 3.